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The Ability of the Saprolegniaceae to Parasitize Platyfish[1]

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(Plate I)

A NUMBER of species of fungi belonging to the Saprolegniaceae have been isolated from naturally occurring infections in fish and other aquatic animals (Table 1). The list is so diverse, from a taxonomic point of view, as to suggest that any saprolegniaceous fungus might be capable of parasitism under appropriate conditions. The experiments reported here were undertaken to determine whether genera and species not previously reported to be parasitic, as well as saprophytic isolates of species known as parasites, could infect platyfish under conditions which allowed infection by two fungi isolated from naturally occurring infections. The platyfish is known to be susceptible to *Saprolegnia* (Gordon, 1936). However, there is no evidence of host specificity for these fungi even though there is variation in the susceptibility of different fish in captivity (Tiffney, 1939a) as well as in nature. Tiffney (1939a) reported that *Saprolegnia parasitica* can attack at least 16 species of fishes and 2 species of amphibians. *Saprolegnia parasitica* also attacks aquatic reptiles both in nature and in captivity. Other species have been reported in crustaceans (Atkins, 1954; Höhnk & Vallin, 1953; Prowse, 1954; Smith, 1940), lamellibranchs (Atkins, 1954), larval mosquitoes (Rioux & Achard, 1956) and rotifers (Valkanov, 1931).

Mycosis caused by Saprolegniaceae is a disease of temperate and tropical freshwater and possibly brackish water fishes. Those affecting temperate water fishes, *e.g.* carp, have been recognized in Central Europe since the Middle Ages. The number of fish species susceptible to infections by Saprolegniaceae has never been properly recorded. From more than 25 years' experience with the diseases of fish, it can be stated without exaggeration that most freshwater fishes are susceptible to infection by some species of Saprolegniaceae, especially under tank and hatchery conditions and following injury of the skin or gills. In fishes, primary infections occur in individuals that have lesions caused by injury. Lesions caused by protozoan and metazoan parasites are the loci of secondary infections. At the New York Aquarium, more than 45 deaths were reported in a single year as being caused by "*Saprolegnia*" (Nigrelli, 1943). A re-check of the Aquarium's records for 1939, 1940 and 1941 showed that 24 species of temperate and tropical freshwater fishes, 4 species of urodeles and 4 species of turtles died from such infections. *Saprolegnia*-like infections were also found in the feet of alligators which had developed ulcers from other causes. No efforts were made at the time to determine which species of Saprolegniaceae were involved in these deaths.

The results of our experiments indicate that although the family Saprolegniaceae is primarily saprophytic in nature, the potentiality of parasitic existence is a familial characteristic. Moreover, under our conditions, at least, the infecting fungus can be a primary invader.

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Table 1. Saprolegniaceae Occurring as Parasites of Aquatic Animals

Fungus	Reference
Achlya flagellata	see Tiffney, 1939b
A. polyandra	see Tiffney, 1939b
A. prolifera	see Tiffney, 1939a, b
A. racemosa	see Tiffney, 1939b
Achlya sp.	Tiffney, 1939b
Aphanomyces laevis	Smith, 1940
Ap. astaci[1]	see Prowse, 1954
Ap. daphniae[1]	Prowse, 1954
Ap. hydatinae[1]	Valkanov, 1931
Ap. ovidestruens[1]	see Prowse, 1954
Dictyuchus monosporus	Tiffney, 1939b
Leptolegnia baltica	Höhnk & Vallin, 1953
L. caudata	see Coker, 1923
L. marina	Atkins, 1954
Saprolegnia diclina	Rioux & Achard, 1956
S. ferax	see Tiffney, 1939a, b
S. mixta	see Tiffney, 1939b
S. monoica	see Tiffney, 1939a, b
S. parasitica	see Tiffney, 1939a, b
S. torulosa	see Tiffney, 1939b

[1] If these are indeed valid species.

Materials and Methods

The 19 isolates of saprolegniaceous fungi used are listed in Table 2. *Brevilegnia unisperma* and *Dictyuchus monosporus* were originally included in this group, but were omitted when they failed to produce zoospores under the conditions of the experiments, since infection in nature is normally accomplished by zoospores (Tiffney, 1939a). Two of the fungi listed were isolated from naturally infected aquarium fish: *Achlya* sp. from an electric catfish *(Malapterurus electricus)* and *Saprolegnia parasitica* from the mosquito fish *(Gambusia affinis).*

The *Achlya* sp. was similar to the sterile *Achlya* described by Tiffney (1939b) from the shell of *Chelydra serpentina,* except in the appearance of the "chlamydospores." In our isolate, these structures were spherical to ovoid, never clavate. They were formed first terminally, later on racemose branches or less frequently in basipetal chains. These "chlamydospores" were shown in our isolate to be oogonia. When mats (grown in pure culture) of *Achlya* sp. were placed in a petri dish in proximity to mats of male (E247) and female (355) thalli of *A. bisexualis,* abundant antheridial initials formed on E247 within three to four hours. After 24 hours, well differentiated oospheres could be seen in the "chlamydospores" of *Achlya* sp. A few eccentric oospores maturing in the oogonia of *Achlya* sp. attested to the completion of the mating reaction with E247. There was no reaction between our *Achlya* sp. and the female isolate 355. This fungus cannot, however, be considered a female isolate of *A. bisexualis* or of any other described heterothallic species of *Achlya.* Not only were the majority of oospores aborted in the mating just described, but the characteristic of producing oogonial initials in the absence of hormonal stimuli provided by the presence of antheridial initials is present in no other heterothallic *Achlya* (see Raper, 1955). Since specific distinctions in this family of fungi are made largely on the basis of characteristics of sexual reproduction, we consider it inadvisable to formally describe this species in the absence of a male isolate giving a normal complete mating reaction.

Table 2. The Ability of Various Saprolegniaceae to Infect Platyfish

Fungus Present	Uninfected Fish		Infected Fish	
	Alive	Died	Alive	Died
None	2	2	0	0
None	4	0	0	0
Achlya sp.	1	0	1	2
A. ambisexualis E87 ♂ (J.R.R.)	0	0	0	4
A. ambisexualis 302 ♀ (J.R.R.)	0	0	0	4
A. bisexualis E247 ♂ (J.R.R.)	0	0	0	4
A. bisexualis 355 ♀ (J.R.R.)	0	0	0	4
A. glomerata (A.W.Z.)	2	2	0	0
A. Klebsiana (L.S.)	0	0	0	4
A. Sparrowii[1]	0	0	0	4
Aphanomyces laevis (A.W.Z.)	0	0	0	4
Calyptralegnia achlyoides (L.S.)	0	0	2	2
Isoachlya monilifera	3	0	0	1
Protoachlya paradoxa (L.S.)	0	1	0	3
Saprolegnia delica (L.S.)	0	0	0	4
S. ferax	0	0	0	4
S. megasperma[2]	0	1	0	3
S. mixta	0	0	0	4
S. parasitica	1	0	0	3
Thraustotheca clavata	0	0	0	4
T. primoachlya[2] (A.W.Z.)	0	0	2	2

[1] Johnson (1956) considers this species to be synonymous with *A. racemosa* Hildebrand. Since *A. racemosa* has centric oospores while *A. Sparrowii* has subcentric oospores (a distinction which Johnson considers, in the same study, to be of subgeneric rank), we prefer to retain *A. Sparrowii.*

[2] Two broods of young were born in the course of this experiment. Normal young (9 and 5) remained healthy; premature fry (*i.e.* with visible yolk sac) became parasitized and died (5 and 3). It was the mother of one of these broods that died without becoming parasitized by *Saprolegnia megasperma.*

The Mexican platyfish, *Xiphophorus maculatus,* was used as host in these experiments.

The conditions chosen for these experiments were determined, by means of preliminary experiments, to provide the greatest chance of infection by the two parasitic isolates *(Achlya* sp. and *Saprolegnia parasitica)* together with the lowest mortality from causes other than induced fungal infection. Pyrex kitchen trays (ca. 8″× 12″×2″) were washed thoroughly, steamed, filled with 1.5 liters of tap water, and placed on an illuminated bench at room temperature (approximately 20° during the course of these experiments). An excess of fish food was then placed in them and inoculated with a pure culture of a fungus. When the growth of the fungus on the fish food was producing an abundance of zoospores, four platyfish, roughly evenly distributed as to size and sex, were placed in each tray, together with a few strands of *Nitella.* Just before exposure to the fungi, these fish were injured by scraping the scales from an area approximately 2× 2 mm. on one side of the caudal peduncle. Uninjured fish were not attacked; fish injured by mere rubbing or nicking of the caudal peduncle were not consistently attacked. The fish were then observed until death, at which time they were removed from the tray, examined to verify the identity of the fungus and preserved in formalin (Plate I), or until the disappearance of the fungus from the tray.

Paraffin sections of the diseased peduncle were prepared and stained with haematoxylin-eosin and with Masson's trichrome stain; whole mounts of the skin and scales showing the mycelia were treated with a modification of Mallory's method in which the following stains were used: Harris' haematoxylin, Phloxin B and Stirling's anilin crystal violet. The tissues were then then treated with Gram's iodine and differentiated with several changes of anilin oil until no more color was removed. The stained material was then passed through several changes of xylene and mounted in Permount. With this method, the mycelia stained blue and the sporangia red and the extent of the infection was followed with ease.

Results

The results of these experiments are given in Table 2. It is evident that under the conditions which permit infection by the two parasitic isolates, nearly every saprolegniaceous fungus used can attack, and usually kill, platyfish. The two apparent exceptions, *Achlya glomerata* and *Isoachlya monilifera,* did not in fact present quite the same conditions as the parasitic isolates. *Achlya glomerata* grew sparsely, although what growth there was produced zoospores. *Isoachlya monilifera* grew moderately well before the addition of the fish, which promptly ate up the mycelium, thus greatly reducing their chance of infection. Consumption of mats of living mycelium of *Saprolegnia parasitica* by fish which suffered no harm thereby has been noted by Tiffney (1939a). The fungus appearing on infected fish was in every case the species with which the tray had been inoculated.

The first signs of infection always appeared at the injured area on the caudal peduncle in the form of a tuft of hyphae. Later, in fatal infections, hyphae often emerged from the gills and mouth, and in tufts over the body, or the entire fish became covered with the fuzzy growth of the fungus.

The pathological lesions were more or less similar, varying only in degree. In relatively light infections, the mycelia penetrated the epithelium of the scales and skin with some necrosis of the involved areas. In heavy infections, the epithelium was often sloughed and the scales and fin rays were softened or completely destroyed. The growth penetrated the deeper tissues, the hyphae often passing into the muscle bundles and resulting in hyalinization or complete destruction. Macrophages filled with melanin, cellular debris and blood cells, together with lymphocytes, were invariably found massed in the areas of the mycelial growth. Inflammatory reaction of varying intensity occurred in regions immediately adjacent to the infection. Surprising as it may seem, little or no bacterial infection was present in any of the sections studied, indicating that the tissue destruction was due almost exclusively to the fungi.

Discussion and Conclusions

The criteria listed by Henle in 1840 for establishing a causal relationship between an organism and a disease in its putative host begin with the isolation of the organism from cases of the disease. We have shown that given a set of conditions which allow infection by two saprolegniaceous fungi isolated from diseased fish, all other saprolegniaceous fungi for which the same conditions (including abundant sporulation) could be provided would infect platyfish. Some of the species used, although not isolated from diseased animals in this instance, have previously been reported to be parasitic, *viz. Aphanomyces laevis, Saprolegnia ferax* and *S. mixta.* The remaining 12 species have not been so reported. Since from these results and previous reports at least 27 species in 10 genera of the Saprolegniaceae have been found capable of attacking animal hosts, we may conclude that

potential parasitism is a familial characteristic. But from the ecological point of view the 12 species not known to be involved in natural infections are not parasites. There is, to be sure, a very marked difference in frequency of occurrence of reported parasites. *Saprolegnia parasitica* is universally reported as most common; other species are reported in frequencies ranging down to only a single established case. It would hardly be surprising if continued search should disclose that the forms we have designated as potential parasites are actually parasitic in nature. The reasons for the rarity or absence of the parasitic habit in some species of this family are probably to be found in their specific ecology. A successful parasite in nature must be abundantly sporulating under the conditions of temperature, etc., that exist when and where susceptible animals appear. While the report of Coker (1923) on seasonal occurrence of saprolegniaceous fungi and the extensive studies of Höhnk (1934, 1956), of Höhnk & Bock (1955) and of Bock (1956) are outstanding as contributions to our knowledge of the specific ecology of the Saprolegniaceae, more precise quantitative techniques would be desirable before attempts are made to correlate the production of zoospores and infections by various species of the Saprolegniaceae in nature.

Summary

Eighteen of 19 isolates of saprolegniaceous fungi, including 16 species belonging to 7 genera, infected platyfish having a standardized wound on the caudal peduncle. The infection usually resulted in the death of the fish. Histological examination indicated that tissue destruction was due almost exclusively to the infecting fungus.

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EXPLANATION OF THE PLATE

Plate I

Fig. 1. Platyfish infected with *Isoachyla monilifera* (upper) and *Saprolegnia megasperma* (lower). Preserved in formalin. 2×.